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Corresponding author(s): Raul Mostoslavsky

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	STAR aligner, HTseq (v.0.6.0.), EdgeR, DAVID, Seurat (v.3.1.1.), Monocle (v.2.13.0.)
Data analysis	Prism (v5.0/8.0), FlowJo (v9), ImageJ (1.53a), R (v.3.6.1.), Matlab (2018a)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq data of the tumor subpopulations from mouse cutaneous tumors of Sirt6 WT or cKO animals have been submitted to the Gene Expression Omnibus (GEO) database under accession number GSE115953 (Related to Fig. 2d-f and Extended Data Fig. 4e-h). The scRNA-seq data of tumor-propagating cells from mouse cutaneous tumors of Sirt6 WT or cKO animals have been submitted to the Gene Expression Omnibus (GEO) database under accession number GSE147031 (Related to Fig. 4b-e and Extended Data Fig. 9a-j). There is no restriction on data availability.
Human HNSCC scRNA-seq data from Puram S. et al. is available in GSE103322 (Related to Fig. 4h and Extended Data Fig. 10c-d).
Oncomine and TCGA dataset (Related to Extended Data Fig.1a-c & 2e) is available in cBioportal (cbioportal.org). Cancer Cell Line Encyclopedia data (Related to Extended Data Fig.1e) is available in portals.broadinstitute.org/ccle.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size for in vivo study. Whenever the right genotype pairs (WT and Sirt6 cKO) are available, they were subject to perform experiments. For skin implantation experiment, sample size was determined based on the minimal number of independent biological replicates powered to significantly identify an effect. For in vitro studies, whenever possible, at least three biological replicates have been performed. No sample size calculation was made for in vitro studies.
Data exclusions	For RNA-seq, one sample did not show the corresponding gene enrichment of the two markers used for FACS (a6 integrin and CD34), questioning the purity of the subpopulations, thus it was excluded from the analysis. For isotope tracing experiment in SCC13 cells, one shCtrl sample was excluded after LC-MS since for most of the polar metabolites, this sample did not give any detectable raw peak areas presumably due to technical issue during experiment. For bioluminescence imaging and the following quantification analysis, several data points that showed necrotic tumor area based on the imaging were excluded in a single-blinded manner since they misrepresented tumor growth pattern.
Replication	Most of the experiments were performed in at least three biological replicates. Some experiments were performed in two biological replicates with two experimental replicates. All are clearly mentioned in the Figure legend. All attempts at replication were successful.
Randomization	For in vivo studies, animals were chosen based on the desired genotypes. All animals were subject to the chemical treatment (DMBA/TPA). In experiments with DCA and/or NAC treatment, the animals were randomly assigned to the treatment. All the experiments that need allocation into control vs experimental groups were randomly assigned.
Blinding	For in vivo experiments, investigators were blinded during data collection (experiments and treatments), but were not blinded during data analysis. For all the other experiments including flow cytometry, RNA-seq, isotope-tracing experiment, scRNA-seq, MALDI-MSI, and bioluminescence imaging, investigators who performed the core experiments were singly blinded to sample information.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	For immunohistochemistry, the following primary antibodies were used: anti-SIRT6 (Cell Signaling, #12486) 1:50 for human tissues and 1:100 for mouse tissues, anti-GLUT1 (Abcam, ab40084) 1:200 for human and mouse tissues, anti-PCNA (Santa Cruz, sc-56) 1:500 for mouse tissues, anti-phospho-PDH (Abcam, ab92696) 0.1 µg/ml for mouse tissues, anti-MPC1 (Sigma, HPA045119) 1:100 for mouse tissues, anti-SOX2 (Abcam, ab92424) 1:50 for mouse tissues, anti-MDA (Abcam, ab6463) 1:1000 for mouse tissues, and anti-CD34 (BD sciences, 553731) 1:50 for mouse tissues. For immunofluorescence staining, the following primary antibodies were used: anti-GLUT1 (Abcam, ab40084) 1:200 for mouse tissues, anti-CD34 (BD sciences, 553731) 1:50 for mouse tissues, anti-SOX9 (Millopore, AB5535) 1:2000 for mouse tissues, anti-H3K56Ac (Abcam, ab76307) 1:500 for mouse tissues, anti-Keratin 5 (Covance, PRB-160P) 1:1000 for mouse tissues, and anti-Keratin 10 (Covance, PRB-159P) 1:1000 for mouse tissues. The following secondary antibodies were used: anti-rabbit, anti-mouse, and anti-rat conjugated to AlexaFluor488 (Molecular Probe, 1:500-1:1000), AlexaFluor595 (Molecular Probe 1:500-1:1000), AlexaFluor647 (Molecular Probe 1:400-1:1000), and rhodamin red-X (Jackson ImmunoResearch, 1:500-1:1000). For flow cytometry, the following fluorophore-conjugated antibodies were used: anti-CD34-BV421 (BD sciences, 562608, 1:50) and

anti-CD49f-PE (eBiosciences, 12-0495-81, 1:200), anti-GLUT1-A647 (Abcam, ab195020, 1:100). Propidium iodide (Sigma, P4864, 1:1000) or Zombie NIR fixable viability dye (Biolegend, 423105, 1:100) were used to negatively select live cells.
For Western blot analysis, primary antibodies were used as follows: anti-SIRT6 (Cell signaling #12486), anti-H3K9Ac (Millipore, 07-352), anti-H3K56Ac (Abcam, ab76307), anti-total H3 (Abcam, ab1791), anti-GLUT1 (Abcam, ab40084), anti-PDK1 (Cell signaling, #3820), anti-LDHA (Cell signaling, #2012S), anti-phospho-PDH (Abcam, ab92696), and anti-β-actin (Sigma, A5316).

Validation

All the primary antibodies were validated by the manufacturers.
SIRT6, H3K56Ac, and H3K9Ac antibodies were further validated in the lab using SIRT6 WT and KO cells by Western blot.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HSC2 cells (a gift from Dr. Cyril Benes, originally from Health Science Research Resources Bank Japan), SCC13 cells (a gift from Dr. Paolo Dotto, originally from JG Rheinwald lab), Tumor-associated fibroblasts (a gift from Dr. Salvador Aznar Benitah), Human primary keratinocytes (from CellnTec)
Authentication	None of the cell lines were profiled for authentication.
Mycoplasma contamination	HSC2, SCC13, and tumor-associated fibroblasts were tested negative for mycoplasma contamination. Human primary keratinocytes were tested by the manufacturer and were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	All the animals were highly C57BL/6 stain (>96%). All 8-week-old animals with desired genotypes were subject to the chemical treatment after hair removal. Both sexes were used. For skin implantation assay, NSG mice were used around 6-8 week old and for housing convenience, all were females. All the mice were housed in ambient temperatures (20-23oC) and humidity (40-60%) with 12hr light/12hr dark cycle.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experiments were conducted under the protocol 2019N000111 approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	The tissue samples we used for the IHC analysis of SIRT6 and GLUT1 staining came from the Pathology Dept. at MGH, and the samples were de-identified (hence not consider human subject Research), and thus we do not have any information as to the population characteristics of these samples.
Recruitment	N/A
Ethics oversight	The collection and use of discarded, de-identified tissue was reviewed and approved by the Dana-Farber/Harvard Cancer Center IRB (Protocol #03-204).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For in vivo tumor isolation, the detailed protocol is available in Methods section. Briefly, skin tumors were excised, minced, and digested in 0.5% trypsin with keratinocyte serum-free media for 1.5 hour at 37C on a rotating plate protected from light. For ROS measurement, glucose uptake assay, and apoptosis assay, the desired cell population was collected with trypsinization.
Instrument	FACS AriaII, LSRII, Accuri (BD biosciences)
Software	FACS Diva, FlowJo
Cell population abundance	For in vivo tumor isolation, the proportion of live YFP+ tumor cells were between 50-60%. For all the in vitro analysis, after gating with FSC and SSC, gated cells were mostly around 90% (except the apoptosis assay). For apoptosis assay, only small debris was excluded from the analyses to capture all the dead and dying cells.
Gating strategy	In every experiment, no stain and single color controls were used. For in vivo tumor analysis, all the isotype controls were also used. Live cells were selected by FSC, SSC, and PI exclusion. Keratinocyte-lineage cells were further selected by YFP expression.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.